NORTHRUP EXHIBIT B

LICENSING ASSOCIATE: SAT

CLASS CODE: Englr

UNIVERSITY OF CALIFORNIA, BERKELEY
OFFICE OF TECHNOLOGY LICENSING
INVENTION AND TECHNOLOGY DISCLOSURE

(see instructions on reverse side)

CASE NUMBER: B 92-011

LOG DATE:

COMPLETE ITEMS 1-6 - USE ADDITIONAL SHEETS AS NECESSARY

1. TITLE OF INVENTION

MICROINSTRUMENTATION - BASED POLYMERASE CHAIN REACTION (PCR) DIAGNOSTICS

2. INVENTOR(S)	TITLE	CAMPUS UNIT OR MAILING ADDRESS
M. ALLEN NORTHRUP	POSTDOCTORAL FELLOW (LLNL); VISITING SCHOLAR (UCB)	LLNL; BSAC ↑
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3. CONTRACT OR GRANT NO.(S)	SPONSOR(S)	PRINCIPAL INVESTIGATOR
N/A	—	—

4. EVENTS	DATE	REFERENCES & COMMENTS
A. Initial idea		TELEPHONE CONVERSATION BETWEEN THE INVENTORS
B. First description of complete invention, oral or written (conception)*		THIS DISCLOSURE (ATTACHED)
C. First successful demonstration, if any (first actual reduction to practice)*	NOT YET	
D. First publication containing full description of invention (establishment of publication bar)*	NOT YET	
E. External oral disclosures	NONE	

5. BRIEF ABSTRACT OF INVENTION - ATTACH DETAILED DESCRIPTION

INVENTION CONCERNS APPLICATION OF MICRO-STRUCTURES TO INSTRUMENT PCR AMPLIFICATION OF DNA, ETC. A SPECIFIC EMBODIMENT EMPLOYING THE LAMB-WAVE ULTRASONIC TECHNOLOGY IS SHOWN.

KEYWORDS (OTL USE ONLY)

6. INVENTION SUBMITTED BY:

MAN — Inventor's Signature — Date

RMW R. M. White — Co-Inventor's Signature — Date

/ — Co-Inventor's Signature — Date

/ — Co-Inventor's Signature — Date

INVENTION DISCLOSED AND UNDERSTOOD BY:

Scott A. Taper, Jr. — Witness' Signature** — Date

Scott A. Taper, JR. — Print Name

Reviewed by: Scott A. Taper, Jr. — Licensing Associate — Date

* See instructions on back.

** Please have PI sign if PI is not an inventor.

UCB OTL

Invention Disclosure Statement

Title: Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics

Inventors:

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Date:

Background:

The polymerase chain reaction (PCR) is a method by which a single molecule of DNA (or RNA) of an organism can be selectively amplified several millionfold within a few hours. This well-established procedure is based on the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of the original DNA molecule, specific DNA primers, dNTPS, and DNA polymerase enzymes. Each cycle produces a doubling of the target DNA segment, leading to an exponential accumulation of the target segment. The generalized procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract, 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and two oligonucleotide primers, 3) thermal cycling of the reaction mixture at two or three temperatures (i.e., 94, 72, and 37-54 °C) for typically 20 to 30 cycles, and 4) amplified DNA detection. Intermediate steps are introduced in some assays to incorporate signal-producing and/or surface-binding primers, and to purify the reaction products (e.g., electrophoresis or chromatography. Reaction volumes and times are typically on the order of tens of μLs and one to two hours, respectively. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical diagnostics, and biological research.

Monolithic microfabrication technology has advanced to the point where a variety of of micro-scale components can be made that have electrical, mechanical, optical, chemical, and thermal capabilities. For example, devices have been fabricated that can pump, heat, cool, and mix microliter quantities of solids and liquids. As well, micro-scale optical and electromechanical/chemical physical and chemical sensors have been developed such as fiber optic probes and Lamb-wave sensors. The integration of these devices into systems allows the development of analytical instruments on a micro-scale. The advantages of such integrated devices include the ability to manufacture them in batch quantities with high precision, yet low cost. Their inherent small size also provides significant advantage in that they would be able to perform highly automated *in situ* analyses.

Invention Concept

The invention disclosure herein concerns the application of microinstrumentation to PCR. The small analytical and reaction volumes of PCR make it an ideal diagnostic technique for

implementation on micro-devices. Such a system could contain reservoirs of reagents, agitation and mixing devices to process the target cells, pumps to carry solid and/or fluid reagents to mixing chambers, heaters and coolers to perform the denaturing and annealing cycles, optical and/or electromechanical/chemical sensors to discriminate the reagents and products of the reaction, and separation devices to purify reactants and products. Feedback control via integrated sensors could also be incorporated directly into the system.

Many or all of of these devices could be made from microfabrication technology and could process micro- to picoliter volumes. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-based diagnostics could be devised. A schematic diagram of an example of one such possible system is presented in Figure 1. Several to many of these micro-instruments could be manufactured on a wafer and could run in parallel, allowing the processing and analysis of several target agents and controls. Target DNA detection methodology could include either an optical, electromechanical, electrochemical, or a combination sensing device. Detection signals could be processed and stored with microelectronic devices.

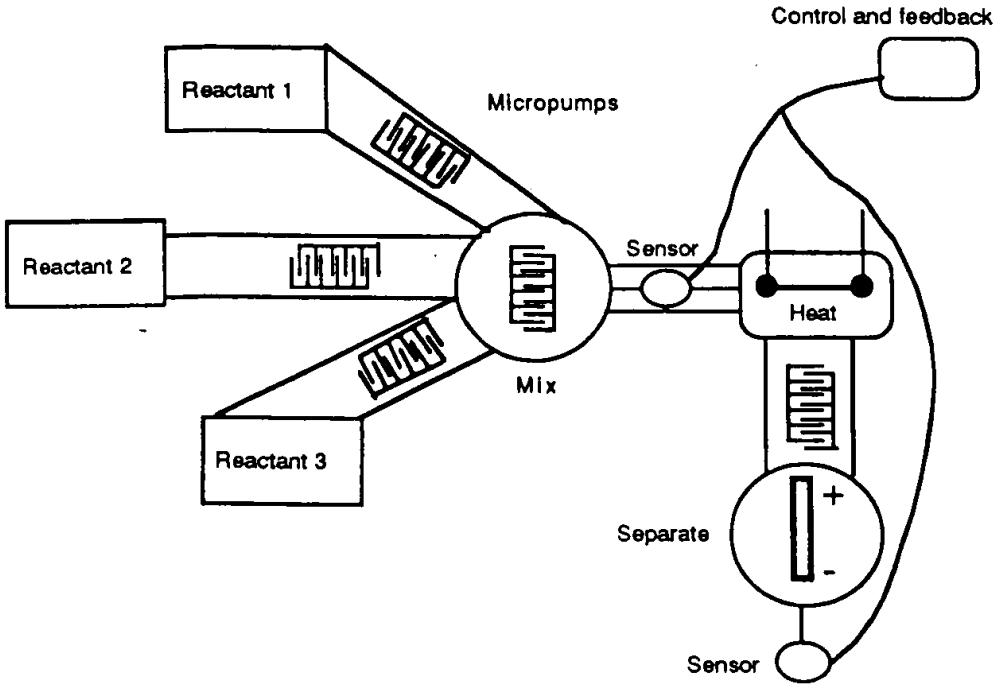

Figure 1. An example of an integrated microinstrument.

In summary, in this disclosure we describe an integrated microsystem and analytical instrument to perform PCR-based diagnostic methodology. The amplification process from minute sample sizes and reaction volumes, and specific reaction sequence of the PCR technique plays favorably into the micro-device capabilities of on-going microfabrication technology. The development of this integrated micro-PCR system will lead to a highly automated, miniaturized, analytical instrument for *in situ* analyses of a variety of samples.

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Witnesses:

Christine Saunders _____ Date _____

_____ Date _____

SUMMARY OF LAMB-WAVE DEVICE TECHNOLOGY

R. M. White --

Device Applications: The basic Lamb-wave device fabricated by planar processing (low-stress LPCVD silicon nitride, RF planar-magnetron sputtered ZnO, aluminum) may be used in three major ways announced to date:

- •1 Physical sensing: Velocity affected (differential pressure, temperature); attenuation affected (fluid viscosity).
- •2 Gravimetric sensing: Velocity affected (absolute gas pressure, fluid density, chemical vapors and gases, protein or bacterial attachment in liquid, diffusion through gels, biochemically selective sensing not yet demonstrated).
- •3 Kinetic effects: In gaseous ambient (moving solid blocks, granular powders); in liquid (pumping fluids, trapping and spinning bacteria, mixing (fluids, and for higher collection efficiency at electrochemical electrodes).

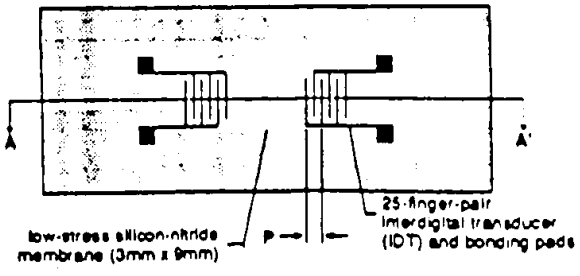
Anticipated Technological Developments: Micro-flow systems (sensing and kinetic functions combined on dies or entire wafers for reconstituting dried biochemicals, chemical synthesis, heat redistribution in IC chips); combination of ultrasonic with optical vapor sensing in single structure; use of polymeric propagation structures offering more geometrical freedom; employment in vapor and biomedical sensing systems with disposable elements likely; use of arrays of devices for selectivity and/or multi-measurand determination; low TEMPCO designs; high-resolution position sensing.

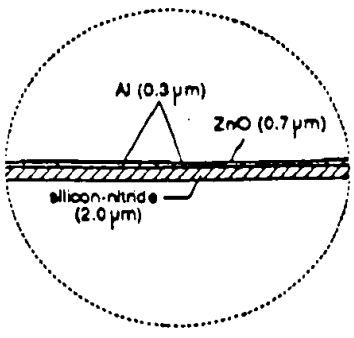
Fabrication Details: Present devices employ two unusual technologies -- deposition of low-stress nitride (to 2 micron thickness) and piezoelectric film (to 1 micron thickness). Feature sizes range from 25 microns downward (thinner membranes and smaller transducer periods lead to higher sensitivities). Devices having transverse dimensions from 3 X 3 cm^2 to 0.15 X 0.05 cm^2 have been made.

Business Aspects: A particular vapor sensing market of 0.5 million devices identified. A fab house might supply basic devices to others who would apply proprietry coatings or special packaging. Patent applications in progress on the technology (U. of California), some of which predates BSAC and so is available for exclusive licensing. Estimated $0.15-0.20 cost per die with dedicated fab. Relationships with BSAC members possible.

Sketches and Specs of Representative Devices:

Operating frequencies of flexural mode: 0.6 - 10 MHz
Gravimetric sensitivity factor: at least 1000 cm^2/g at 2.6 MHz
Short-term noise: approx. 10^{-7} ppm
Estimated minimum detectible added mass/area change: 100 pg/cm^2 water loaded
Differential pressure sensitivity: 0.65 Hz/microbar
Absolute pressure sensitivity: 1.5 kHz/atmosphere (helium)
Viscosity measurement range: from <1 cp to more than 10,000 cp.
Temperature coefficient of delay (uncompensated): -120 ppm/ °C





LAMB-WAVE INTERACTIONS WITH THE CHEMICAL, BIOLOGICAL AND PHYSICAL ENVIRONMENT

R. M. WHITE, S. W. WENZEL, B. A. MARTIN, R. M. MORONEY, B. J. COSTELLO, M. A. STRAUB and R. T. HOWE

Berkeley Sensor & Actuator Center, University of California, Berkeley, CA

Microfabrication processes permit one to form self-supporting micron-thick membranes, in a silicon frame, that support propagating ultrasonic Lamb waves. With membrane thicknesses much smaller than the ultrasonic wavelength, low wave velocities (60-500 m/s) and low operating frequencies (0.6-5 MHz) result. These slow ultrasonic delay lines can be in contact with or immersed in ideal liquids without suffering excessive loss due to radiation. They are also very sensitive to mass loading and can have large wave amplitudes (1000Å with 10-volt drive).

Fig. 1 shows views of a typical device. Composite membrane thicknesses from one to four microns have been utilized, with wavelengths from 40 to 100 micrometers. Tests and/or analyses of four types of functions have been made:

- Sensing physical quantities such as differential pressure and viscosity.
- Non-selective gravimetric sensing of chemical vapors, and proteins or bacteria in a liquid that contacts the sensor.
- Selective ligand-based gravimetric sensing with antigen-antibody binding or DNA probes.
- Kinetic effects such as moving solid particles or bacteria, and mixing of fluids.

Fig. 2 shows responses to water-glycerol soluti having various viscosities. The linear dependen of insertion loss (corrected for density) on viscosity agrees with theoretical predictions. T thicker membranes respond most strongly to viscous dissipation.

Non-selective gravimetric sensing (Fig. 3) has b observed with polymer-coated devices. Analysi shows that the fractional frequency change, whi equals the fractional velocity change, is larger the Lamb-wave gravimetric sensor than for corresponding SAW or bulk-mode sensors. Table lists calculated operating characteristics of a typical device. Note that the minimum calculate detectible mass change per unit area while in contact with water is only 96pg/cm^2. We have successfully detected the non-specific absorpti of protein molecules and bacteria from saline ir contact with the device. Tests of selective liga based sorption are underway.

Large particle motions result because of the confinement of the wave energy in such a thin region. We have moved nanogram-mass polysili blocks with RF drive voltages as low as 1.5 V, a have measured block velocities up to 2 cm/s ca by a 10-volt drive. The waves have produced fl circulation and gently transported biological objects. Mixing experiments in an electrochem cell are in progress. Because of their relatively large amplitudes, it is also possible to observe these waves visually.

We will describe experimental observations and calculated limiting sensitivities in this paper, together with some possible applications of the Lamb-wave devices.

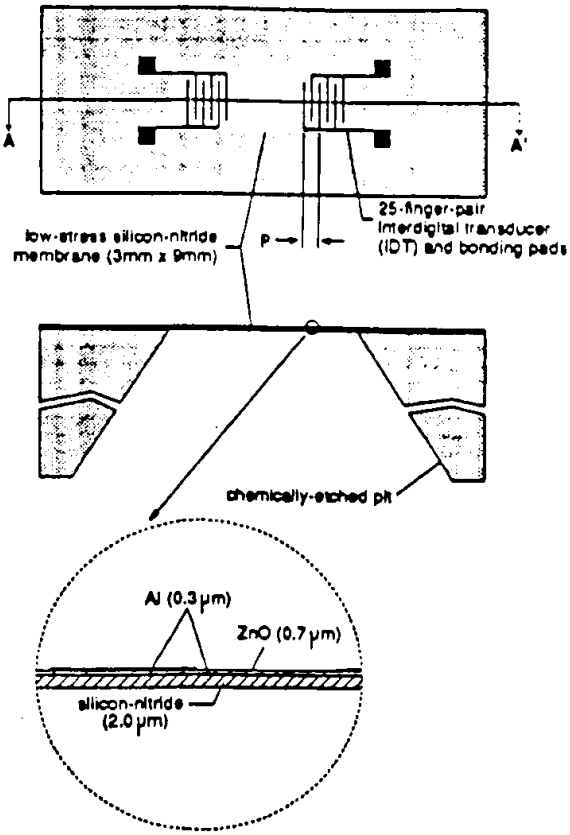


Fig.1. Typical Lamb-wave delay line. With a 100-micrometer wavelength, equal to the period of the interdigital transducers, p, the device shown has a wave velocity in vacuum of 474 m/s and operates at 4.74 MHz.

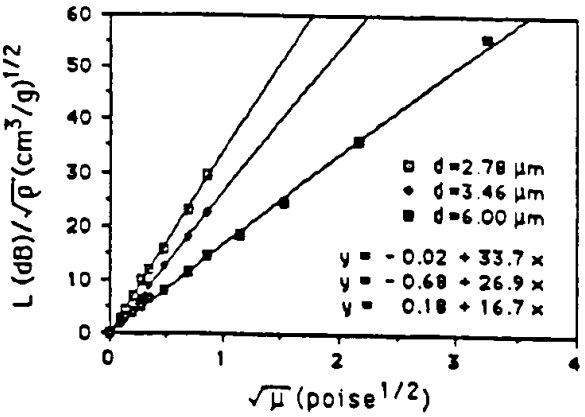


Fig. 2. Insertion loss, L, corrected for density variations, vs. square root of viscosity for devices having three different thicknesses, d.

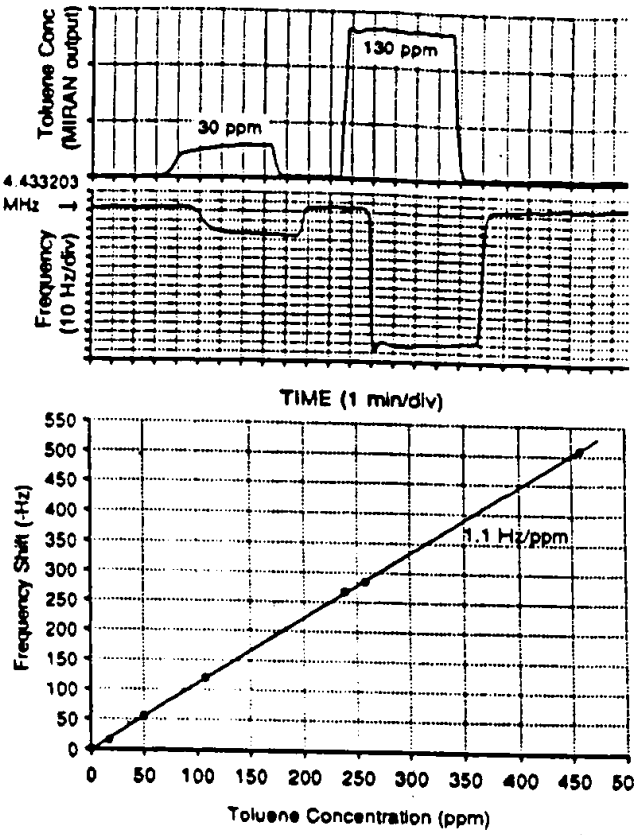


Fig. 3. Toluene vapor sensing with Lamb-wave sensor coated with poly(dimethylsiloxane). Top trace: concentration of toluene in air carrier ga as indicated by MIRAN infrared spectrophotomet Middle trace: frequency of Lamb-wave sensor (o minute delay is caused by gas transport through heat exchanger coils). Bottom trace: Frequency shift vs. toluene concentration. Minimum detectible concentration at 3:1 signal-to-noise ratio is 1 ppm; with ethyl cellulose coating it is only 70 ppb.

DEVICE PARAMETERS:	
Thicknesses:	
*Silicon nitride (um)	0.1
Zinc oxide (um)	0.1
Ground plane (um)	0.1
Total (um)	0.3
Transducer:	
*Period (um)	8
*Mode number	1
Loading conditions:	
*No. loaded surfaces	1
*Fluid sp. gravity	1
*Fluid sp. viscosity	0

OPERATING CHARACTERISTICS:	
*Instability (ppm)	
Instability (Hz)	7.7
Wave velocity (m/s):	
Loaded	309
Frequency (MHz):	
Loaded	38
Mass sensitivity (cm^2/g):	
Unloaded	-43
Loaded	-20
Min. detectible loads (pg/cm^2):	
Mass/area (unloaded)	45
Mass/area (loaded)	96

Table I. Calculated sensitivities of optimized Lamb-wave sensor. Asterisk denotes quantity to set. Dimensions are in micrometers. One surfac of device is assumed to be in contact with non-viscous fluid having density of water ("loaded") in a vacuum ("unloaded"). Mass sensitivity is ra of fractional frequency or velocity shift to adde mass per unit area.

BSAC Lamb Wave References

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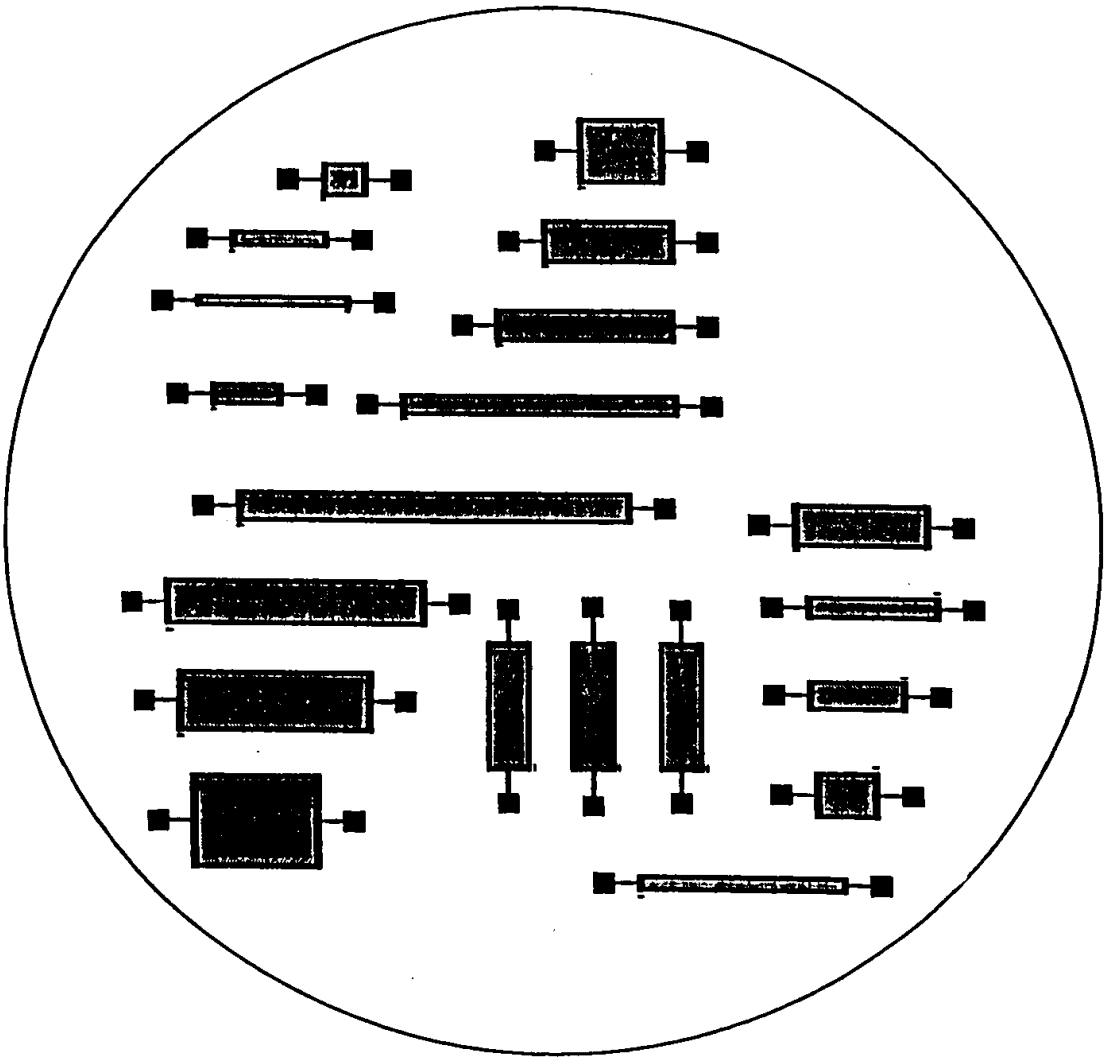
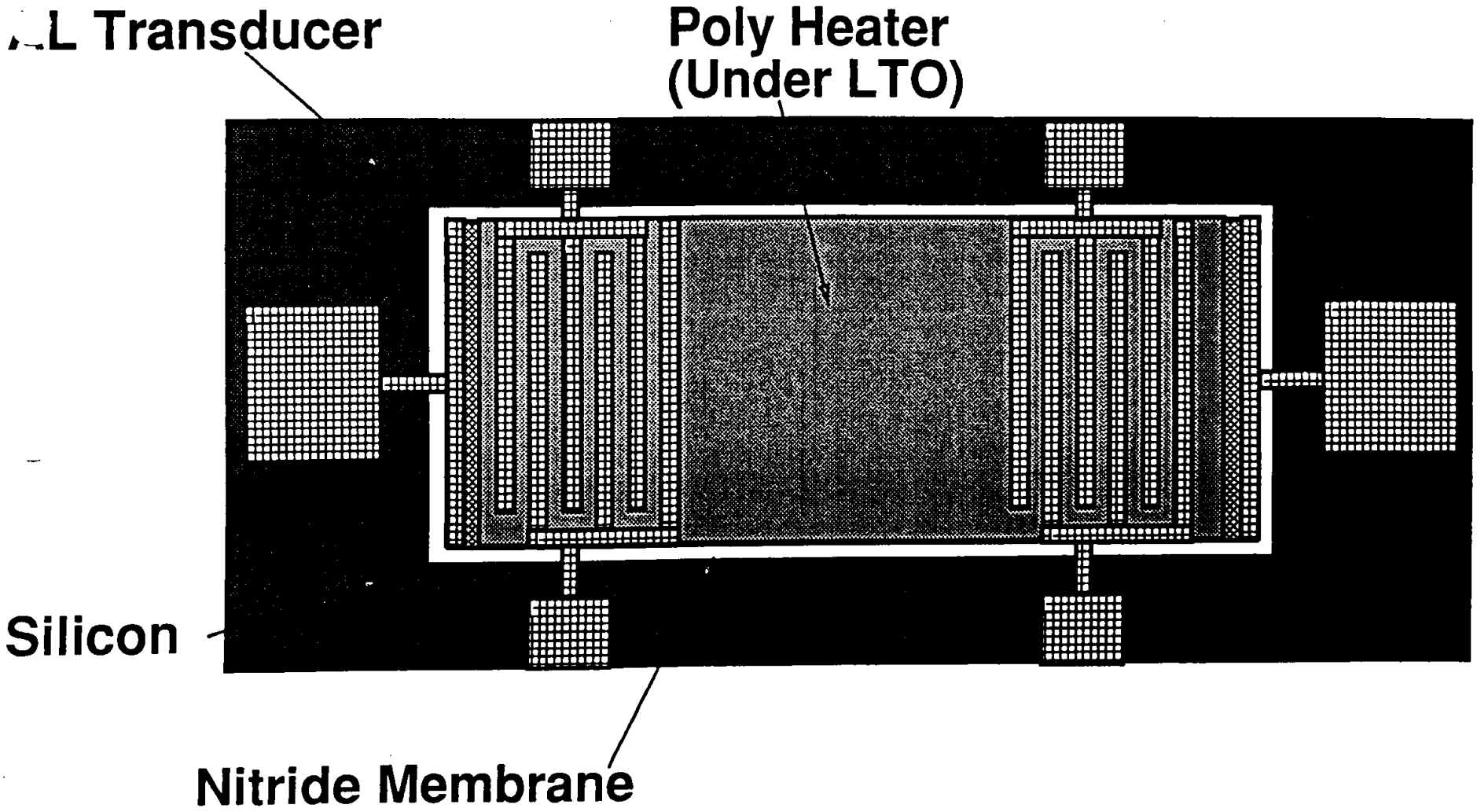
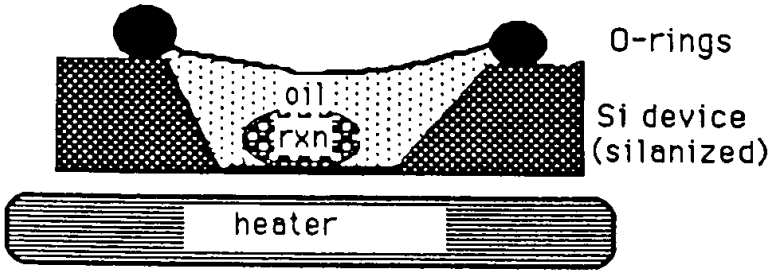
POLYSILICON HEATING ELEMENT TEST STRUCTURES



BIOCHEMICAL MICROFLOW CHAMBER (TOP VIEW)





Example: device volume = 50 µl
reaction volume = 30 µl
oil = 100 µl

BIOCHEMICAL MICROFLOW CHAMBER